J. Physiol. (1980), **308**, *pp.* 353–367
With 11 *text-figures*
Printed in Great Britain

PROPERTIES OF THE CURRENT i_f IN THE SINO-ATRIAL NODE OF THE RABBIT COMPARED WITH THOSE OF THE CURRENT i_{K_2} IN PURKINJE FIBRES

BY DARIO DIFRANCESCO AND CARLOS OJEDA*

From the Istituto di Fisiologia Generale e di Chimica Biologica dell' Universitá di Milano, Via Mangiagalli 32, 20133 Milano, Italy

(*Received 10 December 1979*)

SUMMARY

1. Properties of the 'pace-maker' current i_f in rabbit sino-atrial node have been investigated by voltage clamp of small preparations and compared with those of the i_{K_2} current in the Purkinje fibre. Besides having a similar voltage range of activation and responding in a similar way to adrenaline, i_f resembles i_{K_2} in other respects.

2. When external Na is reduced, i_f decreases proportionally. In 25 % Na the time-dependent current change due to i_f disappears.

3. 20 mM-Cs completely abolishes i_f.

4. The time constant of i_f during a hyperpolarizing voltage-clamp pulse displays a relatively high temperature dependence.

5. In spite of the similarities between the two current systems, experiments in high K solutions (48 mM) rule out the possibility that the current change seen on a hyperpolarization reflects the decay of a pure K current.

6. From conductance measurements during onset of i_f it is deduced that i_f behaves as an inward current activated by hyperpolarizations.

INTRODUCTION

It has recently been shown that the current system i_f present during the pace-maker depolarization phase in the rabbit sino-atrial (S-A) node, and involved in the acceleratory action of adrenaline, appears as a large inward current slowly activating (or outward current deactivating) on hyperpolarizations below -50 to -60 mV from a holding potential of about -40 mV (Brown, DiFrancesco & Noble, 1979). Although the S-A and auriculo-ventricular nodal tissues are the only ones whose normal activity is rhythmic in the heart, natural pace-maker depolarization can also arise in Purkinje fibres where it is governed by the decay of the current i_{K_2} (Noble & Tsien, 1968; Peper & Trautwein, 1969). As observed in the preceding paper (Brown & DiFrancesco, 1980), besides both acting as 'pace-maker' currents, the two current systems i_f and i_{K_2} are similar in other respects, as follows.

(1) the voltage range where they are observed on voltage-clamp hyperpolarizations from a low holding potential is the same (more negative than -50 to -60 mV).

* Present address: Inserm 121, Hôpital Cardiovasculaire, 22 Avenue Du Doyen Lepine, 69500 Bron-Lyon, France.

0022-3751/80/2520-0655 $07.50

(2) Substantial activation of i_f during a hyperpolarizing pulse is followed by a much smaller current tail on return to the holding potential (see Figs. 2–9 of this paper). This is also a property of i_{K_2}, whose fully-activated $I-V$ relation displays a marked inward-going rectification (Noble & Tsien, 1968). (3) By shifting the s_∞ curve to more positive potentials, adrenaline increases the amount of i_{K_2} activated by a hyperpolarization from about -40 mV, as observed for i_f (Brown *et al.* 1979).

In this paper the properties of i_f in comparison with those of i_{K_2} are investigated in more detail. Remarkable analogies with i_{K_2} are found in the dependence of i_f upon Cs and Na removal. Also the i_f time constant displays a relatively high temperature dependence (Q_{10} varying from 2–4), though not as high as that found for i_{K_2} ($Q_{10} = 6$ according to Noble & Tsien, 1968 and 17 according to Cohen, Daut & Noble, 1976). However, while it seems well established that i_{K_2} is carried by K, we also present evidence that the way i_f depends on external K seems to exclude its being a pure K current. Furthermore membrane conductance measurements during onset of i_f on hyperpolarizing pulses demonstrate that i_f behaves as an inward current which increases with time during a negative pulse and is activated at potentials more negative than -50 to -60 mV.

METHODS

The methods used are essentially the same as those described in the preceding paper (Brown & DiFrancesco, 1980). In brief, strips of tissue from the nodal region of the right atrium were dissected and immersed in Tyrode solution at about 35 °C. The size of a beating preparation was reduced by progressive cuts and ligatures down to about 250×250 μm. Voltage uniformity of the preparation was controlled by checking that no phase shift was present between action potential upstrokes in two different cells. Voltage clamp was performed by traditional methods. Standard 3 M-KCl micro-electrodes were used with resistances ranging from 20–40 MΩ. Voltage and current traces were recorded on a two-trace storage oscilloscope and on a four-channel FM tape recorder for subsequent analysis. Tyrode solution was composed as follows (mM): NaCl, 140; KCl, 3; $CaCl_2$, 1·8; $MgCl_2$, 1. The solution was buffered with Tris and the pH adjusted to 7·2 by adding HCl. In the Na-removal experiments Na was replaced by equimolar quantities of Tris. CsCl and KCl were added to the control solution without correcting for the change in total osmolarity. The control solution often contained 5 mM-$MnCl_2$ and TTX 5×10^{-7}–10^{-6} g/ml. in order to avoid interference from inward currents during depolarizing voltage-step pulses. The presence of agents which block the slow and fast inward currents improved the voltage control during a positive step pulse.

The current i_f studied in this paper appears during a hyperpolarizing voltage-clamp pulse as a time-dependent change which is either the time-dependent increase of an inward current or the time-dependent decrease of an outward current. Although it is not until the end of the Results section that we shall have presented enough evidence to decide which of these two possibilities is the more likely, for clarity we will adopt from the start the convention of referring to the time-dependent change during a hyperpolarization as the 'activation' of i_f.

RESULTS

Effects of low-Na solutions on i_f

The activation of the i_{K_2} channel in Purkinje fibres is known to depend on external Na (McAllister & Noble, 1966). DiFrancesco & McNaughton (1979) find that the i_{K_2} channel behaves as if external Na were necessary to activate it, but no satisfactory explanation has been found for this phenomenon so far. The experiments of Figs. 1 and 2 demonstrate that the current i_f too depends on the presence of external Na.

Fig. 1 shows the effect on i_f of 50 % Na replacement by Tris, during hyperpolarizations in the range −47 to −77 mV.

The time-dependent i_f change seen in the control case (*A*) is markedly reduced after 5 min perfusion with 50 % Na (*B*) and this effect is reversed by returning to the control solution (*C*). The decrease in i_f is accompanied by an increase of the holding current in the outward direction, probably reflecting a diminished contribution of a background component, and an increase in the time-independent current jump at the on and off of pulses. Further reduction in external Na causes i_f to diminish further, as shown in Fig. 2. Here the current traces recorded on a 800 msec hyperpolarization from −37 to −72 mV are superimposed at various times during a change from control to 25 % Na-containing solution. It is interesting to note that after 4 min the direction of time-dependent change is apparently inverted (trace *c* in Fig. 2). It is

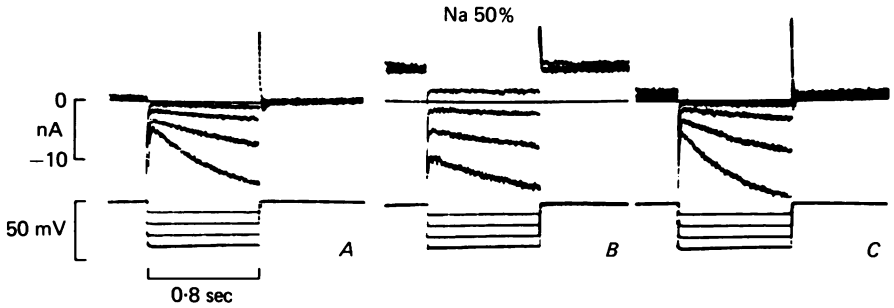

Fig. 1. Effect of 50 % Na reduction on i_f. A set of hyperpolarizing pulses from a holding potential of −47 mV is applied in the control solution (*A*), 5 min after perfusion with low-Na solution (*B*), and 6 min after return (*C*). Control solution contains 5 mM-$MnCl_2$ and TTX 5×10^{-7} g/ml.

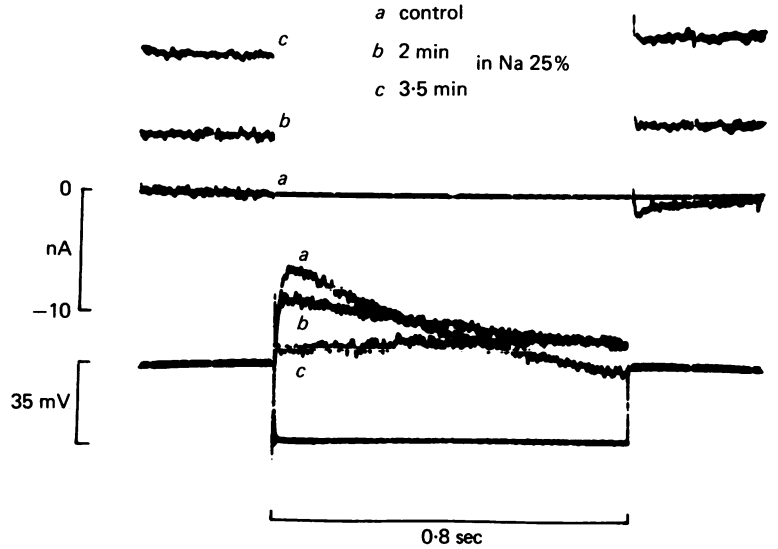

Fig. 2. Current recording during a 35 mV hyperpolarizing pulse before (*a*) and during perfusion with 25 % Na-containing solution (2 min (*b*) and 3·5 min (*c*) after change of solution). Holding potential −47 mV. i_f progressively decreases and eventually becomes an apparent inward-decreasing trace (*c*). Same preparation as in Fig. 1.

difficult to assess, however, to what extent the time course of current in this case is affected by K depletion, the contribution of which is in fact an inward-decaying component during a negative pulse (DiFrancesco, Ohba & Ojeda, 1979).

Abolition of i_f by Cs

Caesium blocks the inward rectifying K channel in skeletal muscle (Beaugé, Medici & Sjodin, 1973) and reduces K permeability in squid axon (Adelman & French, 1978), eel electroplaque (Ruiz-Manresa, Ruarte, Schwartz & Grundfest, 1970) and egg cells (Hagiwara & Takahashi, 1974). Also, Cs has been shown to greatly reduce the currents i_{K_1} and i_{K_2} in Purkinje fibres in concentrations up to 20 mM (Isenberg, 1977). The effect of 20 mM-Cs on the current i_f is shown in Fig. 3.

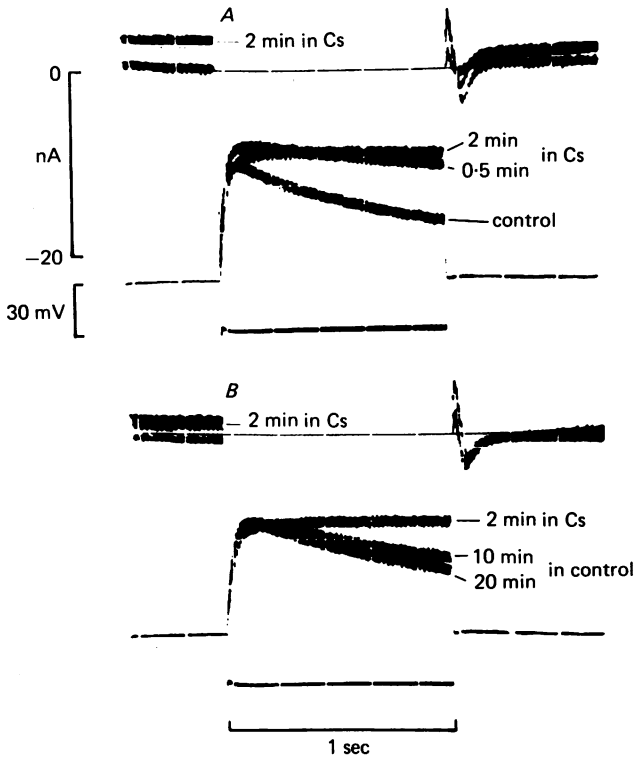

Fig. 3. Block of i_f in 20 mM-CsCl. The preparation is perfused for 2 min with Cs (*A*), and after this time the control solution is readmitted (*B*). 2 min is sufficient to remove completely the time-dependent change due to i_f observed on a 30 mV hyperpolarizing pulse from a holding potential of −38 mV. A much longer time (20 min) is required for nearly complete reactivation of i_f. Control solution contains 5 mM-$MnCl_2$ and TTX 10^{-6} g/ml.

In *A*, perfusion with 20 mM-Cs causes i_f to be completely abolished within 2 min. If after this time the solution is changed back to normal, i_f reappears almost completely (Fig. 3*B*) within 20 min. Fig. 4 shows another experiment where a set of records in the range −48 to −78 mV was taken in the control solution (*A*), 3 min after 20 mM-Cs (*B*) and 30 min after return to control (*C*). The time-dependent

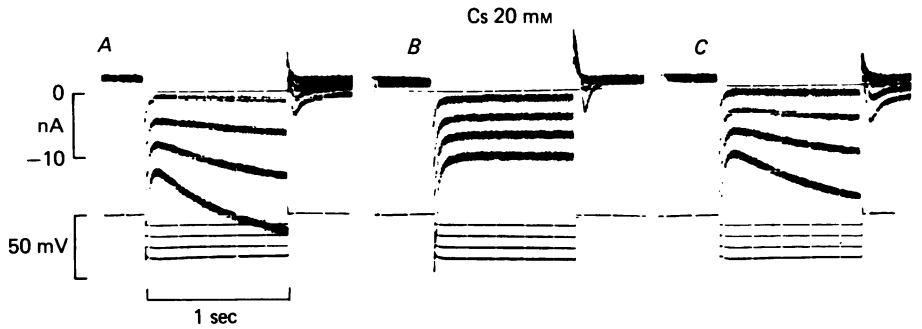

Fig. 4. Effect of 20 mM-CsCl on i_f in the potential range from −48 to −78 mV. The middle traces are taken 3 min after perfusion with Cs, and the right-hand traces 30 min after return to control. Same preparation as in Fig. 3 (second perfusion with Cs).

change of current completely disappears in all the voltage range investigated (together with a reduction in tail amplitude) and is nearly completely reversed after return to control.

Effects of temperature changes on i_f

Raising the temperature increases i_f, an effect which could be responsible for the acceleration of the pace-maker depolarization during spontaneous activity (Brown & DiFrancesco, 1980). In Fig. 5 the same set of hyperpolarizing pulses from a holding potential of −39 mV has been applied to the preparation at three different temperatures. The current i_f is seen to undergo a large increase on warming from 35 to 40 °C, and to be strongly reduced by lowering the temperature to about 27 °C. In this experiment the steady state for i_f was not reached during the pulses, their duration

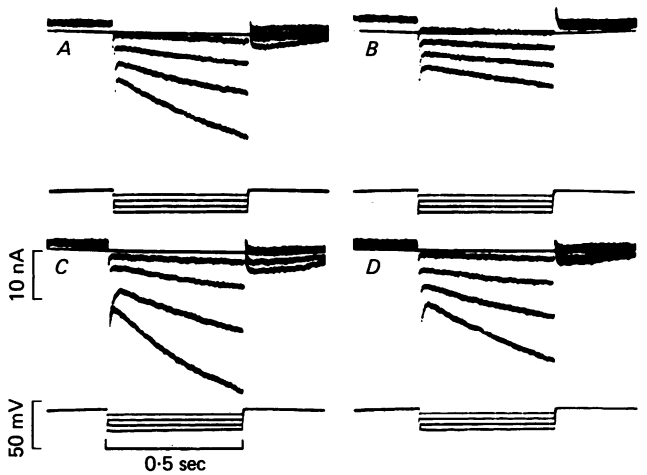

Fig. 5. Effects of temperature changes on i_f. The same voltage protocol is applied at 35·1 °C (*A*), 27·2 °C (*B*), 40·1 °C (*C*) and on return to 34·9 °C (*D*) (control). Holding potential is −39 mV and hyperpolarizing pulses 0·5 sec long are given in 5 mV steps down to −69 mV. Note that the decrease (increase) of i_f at low (high) temperature is followed by a proportional decrease (increase) of i_f tails on return to the holding potential. 5 mM-$MnCl_2$ present throughout the experiment.

being only 1 sec, and therefore it is difficult to assess whether the high temperature dependence of i_f during a hyperpolarization is due to a change in the time constant, or to a change in the total steady-state current, or both.

Results from an experiment where longer pulses were used are shown in Fig. 6. Here two cases are illustrated, in which a 15 mV (*A*) or a 25 mV (*B*) pulse is applied to activate i_f at different temperatures. The current traces are fitted by single exponential curves with time constants of 6·54 sec and 3·62 sec at 35·3 and 39·6 °C respectively at −55 mV, and of 5·49 sec and 9·11 sec at 34·0 and 27·3 °C respectively at −65 mV (Fig. 6*C* and *D*). Q_{10} values of 3·96 and 2·13 are obtained from these results for the 15 mV and the 25 mV pulse. It is clear from Fig. 6 that also the current amplitude is affected by temperature changes. The large difference between the two Q_{10} values could be caused by the different temperature ranges analysed, or simply reflect experimental scatter of points.

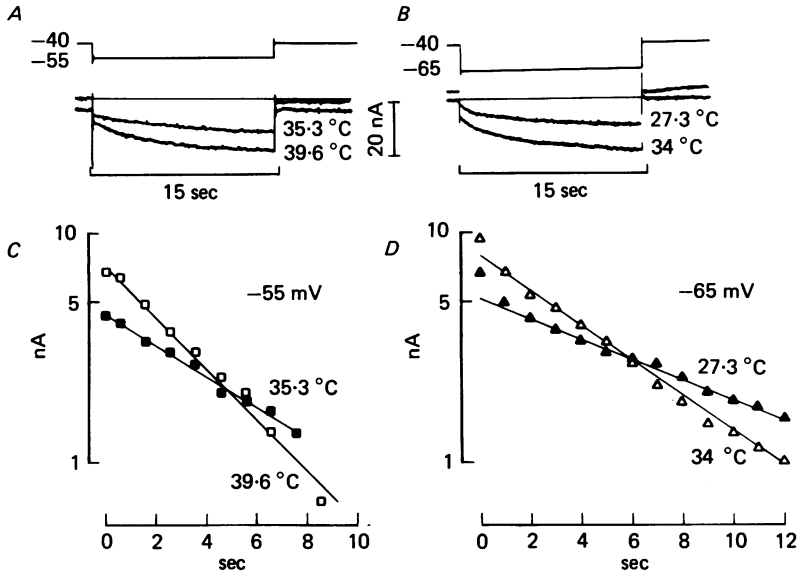

Fig. 6. Time course of i_f at different temperatures. From the holding potential of −40 mV a 15 mV hyperpolarization at the temperatures of 35·3 and 39·6 °C (*A*), and a 25 mV hyperpolarization at 34·0 and 27·3 °C (*B*) are applied. The corresponding current records plotted on a semilog scale against time (*C* and *D*) show a good linearity. The time constants obtained by fitting the traces with the least-squares method are: 6·54 sec and 3·62 sec for the 15 mV pulse records at 35·3 and 39·6 °C respectively, giving a Q_{10} = 3·96; 5·49 sec and 9·11 sec for the 25 mV pulse records at 34·0 °C and 27·3 °C respectively, giving a Q_{10} = 2·13. Tyrode solution containing 5 mM-$MnCl_2$.

Dependence of i_f on external K

As shown above, the response to low Na and to Cs of i_f as well as its range of activation and its response to adrenaline, are similar to those of i_{K_2} and suggest therefore that the two could be identical current systems. A crucial test of this hypothesis is to check how i_f behaves when external K is changed. As shown already by Brown & DiFrancesco (1980), increasing K from 3 to 9 mM leads to an increase in i_f in the range −50 to −70 mV. This is unexpected for a K current, unless its fully

activated *I–V* relations at 3, 6 and 9 mM–K display reciprocal cross-overs at potentials negative to the potential range explored (i.e. more negative than −70 mV). Even if this were the case, further increasing the K concentration should eventually lead to a decrease of i_f, and to its reversal. We have therefore extended the analysis to higher K concentrations, and the results confirm that the behaviour of i_f is not that expected for a K current. In Fig. 7 the K concentration was increased from 3 to 24 mM. Together with an increase in the inward background and in the time-independent components, on raising K a large increase of i_f is apparent throughout the voltage range investigated.

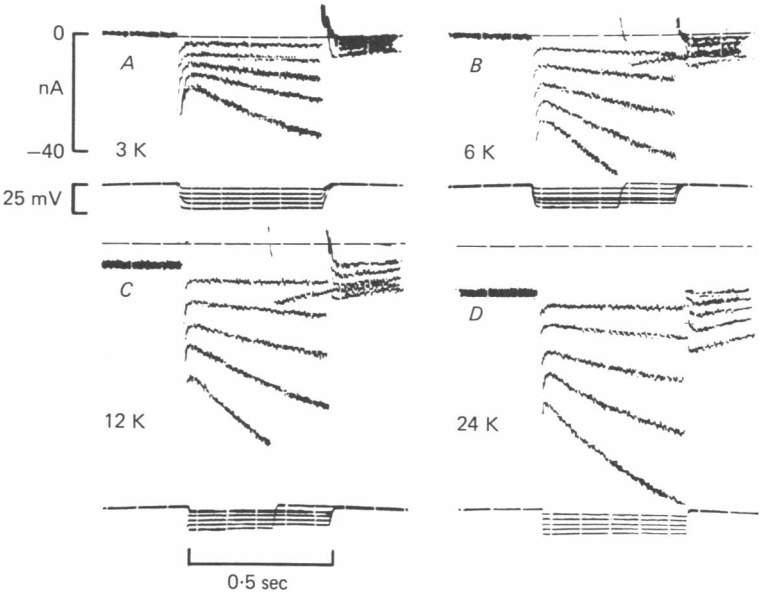

Fig. 7. Effect of external K on i_f. Activation of i_f is investigated in the range −52 to −72 mV from a holding potential of −47 mV in four different K concentrations: 3 mM (*A*), 6 mM (*B*), 12 mM (*C*) and 24 mM (*D*). In all K concentrations i_f increases in the inward direction with increasing negative pulse amplitudes. In addition, at a fixed potential i_f increases on raising K, over all the voltage range analysed. The increase in i_f is accompanied by a displacement of the reference current level in the inward direction. Note also the augmented i_f tails on return to the holding potential when K is increased. All solutions containing 5 mM-$MnCl_2$. At 6 mM- and 12 mM-K the largest hyperpolarization lasted 300 msec only.

In Fig. 8 another experiment is illustrated where K was changed from 3 to 48 mM. At this concentration the K-dependent component i_K (DiFrancesco, Noma & Trautwein, 1979) can be separated from i_f in the voltage range explored. Whereas both i_K and i_f appear as decaying outward currents when hyperpolarizing pulses are applied from −55 to −90 mV in 3 mM-K (Fig. 8*A*), a clear biphasic time course becomes evident in 48 mM-K (*B*) at potentials negative to −65 mV. To show the reversal of i_K at a low membrane potential more clearly the protocol of Fig. 8*C* has been used. A pulse to −55 mV is preceded by a series of depolarizing and hyperpolarizing steps, in order to activate various degrees of i_K. As expected, each pre-depolarization gives rise to a sharp inwardly directed current decay during the following pulse to −55 mV.

It is worth noting that no i_f appears at this potential in these records. This experiment thus provides further evidence that the two components i_K and i_f are separate entities: in 48 mM-K the K-dependent component is observed to reverse positive to -55 mV, while i_f still increases in the inward direction at this potential.

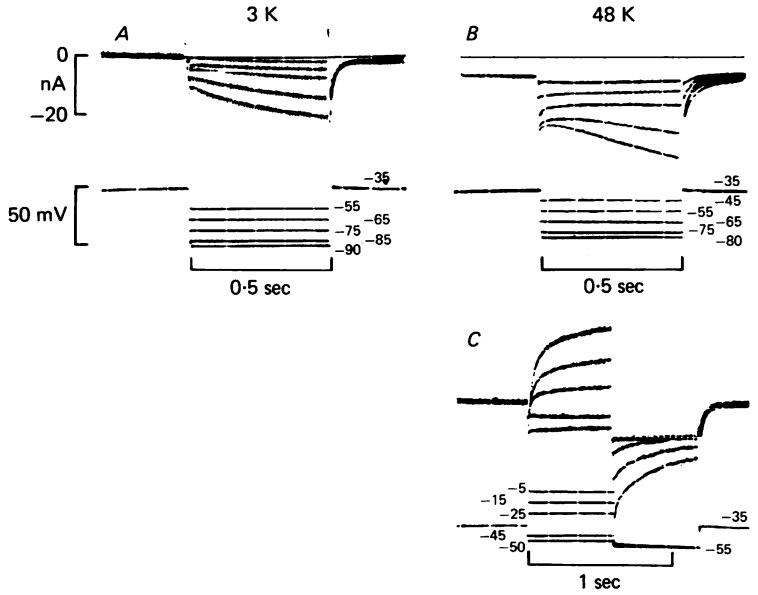

Fig. 8. Separation of i_f from i_K in high K. The same set of 500 msec hyperpolarizations from the holding potential of -35 mV is applied in 3 mM-K (A) and 48 mM-K (B). In the latter case the voltage range investigated extends over values at which i_K is already reversed, as apparent from the early part of current traces in B which show an inward-decaying time course. The reversal of i_K at a potential more positive than -55 mV becomes more evident by giving prepulses to activate i_K to various degrees before the test hyperpolarization to -55 mV (C). After these prepulses i_K appears as inward-decaying. Despite the reversal of the K-dependent current i_K, in 48 mM-K, i_f still increases in the inward direction at potentials as negative as -80 mV (B). Solutions containing 5 mM-$MnCl_2$.

Conductance measurements during i_f onset

A possible way of distinguishing an outward K current which decays from an inward current which activates during a hyperpolarizing pulse, is to measure the membrane conductance during the current change. The experiment of Fig. 9 illustrates how this can be achieved. In general, small and short pulses are superimposed on the main test pulse during which a time-dependent current change is activated. Suppose this current $i(E, t)$ can be expressed during a voltage-clamp to the potential E by the general Hodgkin–Huxley equation

$$i(E, t) = y(E, t)\, \bar{i}(E) \tag{1}$$

where $y(E, t)$ is the activation variable and $\bar{i}(E)$ the fully activated current; then calling δi the instantaneous current jump associated with the small and short pulses of amplitude δE, this will be given by:

$$\delta i(E, t) = y(E, t)\,(\bar{i}(E+\delta E) - \bar{i}(E)) = y(E, t)\,(\mathrm{d}\bar{i}/\mathrm{d}E)_E \delta E \tag{2}$$

Thus supposing in the simplest of cases that the observed total current displacement (δi_t) is the sum of δi and a time-independent contribution, the time course of δi_t yields information on the slope conductance of the fully activated I–V relationship ($\bar{i}$). For example, in the experiment of Fig. 9*A*, depolarizing pulses are given from a holding potential of -35 mV in 48 mM-K. The increasing outward current observed in this case represents the activation of i_K. We therefore expect that the current displacement δi, reflecting a channel opening process, will increase with time, as in fact it does. In Fig. 9*B*, on the other hand, the current displacement δi observed

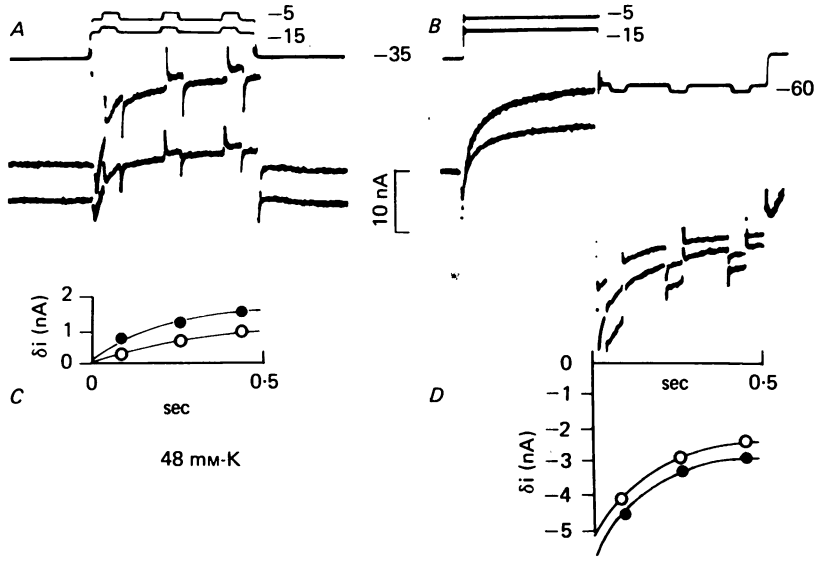

Fig. 9. Membrane conductance measurements in 48 mM-K in the range of activation of i_K. i_K is activated by pulsing positive to the holding potential of -35 mV (*A*) or deactivated by pulsing negative to it (*B*). As the i_K reversal potential is more positive than -55 mV in 48 mM-K (compare with Fig. 9), in the latter case the decay of i_K at -60 mV (test hyperpolarization in *B*) appears as an inward decreasing current tail, whose amplitude increases if a pre-depolarization is given before the test pulse. In *C* and *D* the time course of the current displacement δi associated with the small pulses indicates a channel-opening process in *C*, and a channel-closing process in *D*, as expected from the kinetics of the current in this case. Same experiment as in Fig. 8.

on hyperpolarizing from -5 and -15 mV to -60 mV decreases with time, indicating a membrane conductance decrease and thus a channel closing process. It is worth noting that this happens in spite of an increase of current in the outward direction, simply because the reversal potential of i_K is positive to -60 mV in 48 mM-K (see Fig. 8*B* and *C*) and thus i_K is inward at -60 mV. By the experiment of Fig. 9 the validity of measuring the instantaneous slope conductance is therefore verified in a region where a *decrease* of membrane K conductance corresponds to an increase of current in the outward direction.

Having checked the method on the current i_K, we can now proceed to investigate the time course of the instantaneous membrane conductance during activation of i_f. We expect that if the current change elicited by hyperpolarizations is attributable to a decaying outward current, δi will *decrease* with time, but an *increase* will be observed if instead the current is an increasing inward current. The experiment

illustrated in Fig. 10*A* shows that the latter is in fact the case. The absolute magnitude of δi clearly increases with time during the 20 sec pulses from the holding potential of −39 mV to −59 and −54 mV, and it decreases during the subsequent 10 sec depolarizing steps of 10 mV. This is more evident from Fig. 10*B* where the time course of δi is plotted for the two pulses. It is interesting to observe that the time course of δi should parallel the total current time course if i_f were a HH-type current described by the general equation (1). In this case in fact, calling Δi and $\Delta\delta i$

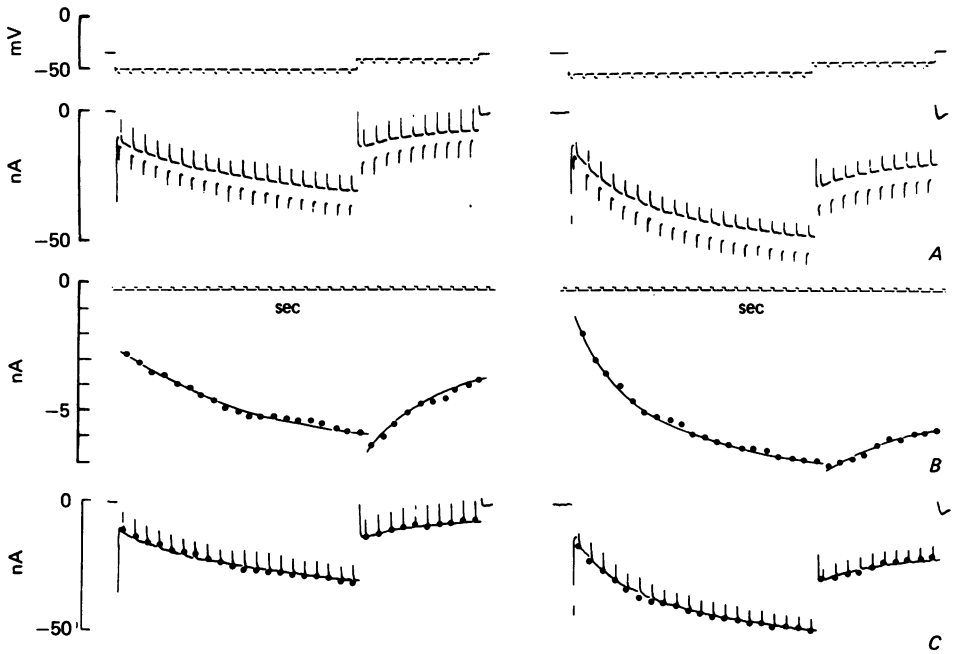

Fig. 10. Conductance measurement during onset of i_f. *A*, test hyperpolarizations of 15 mV (left) and 20 mV (right) are given from a holding potential of −39 mV in order to activate i_f, and are followed by 10 mV depolarizations to deactivate i_f. *B*, plots of the time course of the current displacement δi associated with the small pulses indicate a clear conductance increase during the hyperpolarizations, followed by a conductance decrease during the depolarizations. *C*, the upper part of the total current traces have been redrawn together with the $i(t)$ traces (●), scaled up and shifted for comparison. The scaling factors for the four pulses are (left to right): 6·95, 2·42, 6·57 and 5·76.

the total current and the current displacement referred to their value at time zero (start of the test pulse), from (1) and (2)

$$\Delta i(E, t) = \Delta y(E, t)\, \bar{i}(E)$$
$$\Delta\delta i(E, t) = \Delta y(E, t)\, (\mathrm{d}\bar{i}/\mathrm{d}E)_E\, \delta E,$$

where $\Delta y(E, t) = y(E, t) - y_0$ is the value of the kinetic variable referred to its starting value, y_0. Dividing the two equations side by side we obtain:

$$\frac{\Delta\delta i/\delta E}{\Delta i} = \frac{(\mathrm{d}\bar{i}/\mathrm{d}E)_E}{\bar{i}(E)}$$

and this ratio is only dependent on the membrane potential E. This means that the time dependence of $\Delta\delta i(t)$ and $\Delta i(t)$ should be the same. In Fig. 10*C* the time courses

of Δi and $\Delta \delta i$ are plotted after scaling up the traces of $\Delta \delta i$ for comparison. Within experimental error these are coincident, which suggests that the behaviour of i_f is consistent with the description by means of eqn. (1).

The same arguments which have been given in presenting the results shown in Fig. 7 apply to the time course of δi shown in Fig. 10. Thus if the $I-V$ relationship of i_f had, in the voltage range investigated, a negative slope conductance in 3 mM-K (as has i_{K_2}) a channel-closing process would lead paradoxically to a time-dependent conductance increase. We have therefore checked the time course of δi at high K concentration, where the presence of a negative slope conductance for the $I-V$ relation of an i_{K_2}-type current at about -50 to -60 mV can be ruled out. The result is shown in Fig. 11.

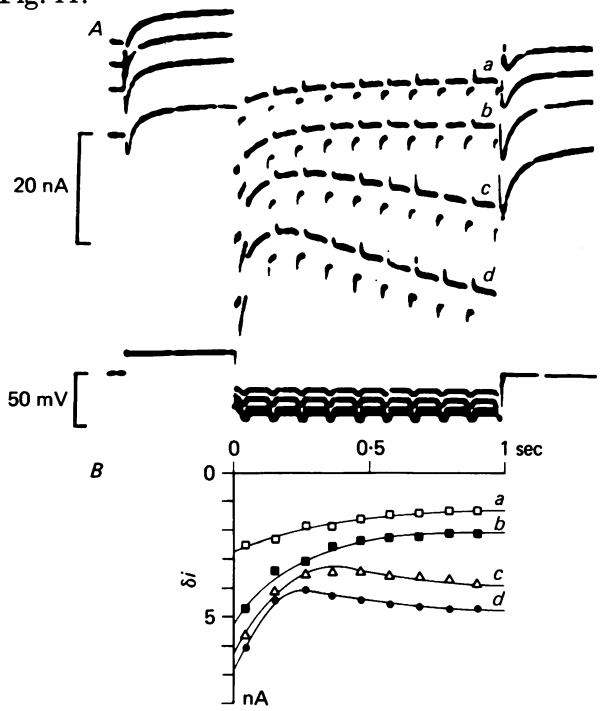

Fig. 11. Comparison between membrane conductance time course during decay of i_K and during onset of i_f. *A*, after a 400 msec, 20 mV pre-depolarization from a holding potential of -35 mV, hyperpolarizing test pulses are given in the range -55 to -80 mV in 48 mM-K. The current time course reflects the deactivation of i_K for small hyperpolarizations (traces *a* and *b*), while the onset of i_f appears at more negative test pulses (traces *c* and *d*). The early part of traces *c* and *d* is inward-decaying and reflects the relatively fast i_K decay. *B*, the time course of the current displacement δi associated with the small pulses shows a conductance decrease for the traces *a* and *b* and for the early part of traces *c* and *d*, as expected in the case of a channel-closing process. In contrast to this, the onset of i_f is associated with an increase with time of membrane conductance, as expected from a channel-opening process. Same experiment as in Figs. 8 and 9.

Here a series of test pulses in the range -55 to -80 mV, preceded by 200 msec pre-depolarizations to -15 mV in order to activate i_K, is given in a solution containing 48 mM-K. At this concentration a clear distinction between the two current systems is visible since they change with time in opposite directions. Thus during the test pulses to -55 and -65 mV the total current changes reflect the inward decay of

i_K (Fig. 11*A*, traces *a* and *b*), while a biphasic time course appears during the pulses to −75 and −80 mV (traces *c* and *d*) in correspondence with the onset of i_f activation. During these latter pulses the current trace is inward decaying for about the first 200 msec (i_K decay) and after this it is inward increasing (i_f onset).

The possibility that this initial inward deflexion represents a depletion process (Baumgarten & Isenberg, 1977; DiFrancesco, Ohba & Ojeda, 1979) can be ruled out, as in 48 mM-K change in the K driving force caused by hyperpolarization is probably negligible. The time course of the magnitude of the current displacement δi is also obtained in Fig. 11*B*. While δi undergoes a reduction during the two lower pulses (*a* and *b*), a clear biphasic behaviour appears during the pulses *c* and *d*, where i_f activation is present. Thus, δi first *declines* with time as it decays, and then *increases* again when i_f takes over. We see therefore that even at very high K concentrations the current displacement δi associated with i_f onset increases with time, and therefore the possibility that the current change during a hyperpolarizing pulse is a decaying K current can be ruled out. On the contrary, the conductance increase during onset of i_f suggests that i_f could be an inward current activated by hyperpolarizations.

DISCUSSION

Similarities between the current system i_f in the S-A node and i_{K_2} in the Purkinje fibre

The current system i_f in the S-A node has been described recently as underlying the pace-maker depolarization and mediating the acceleratory effect of adrenaline (Brown *et al.* 1979). In many respects, as pointed out in a previous paper (Brown & DiFrancesco, 1980) i_f shows marked similarities to another current system, the current i_{K_2} of the Purkinje fibre of the sheep, which has been very fully studied over the past decade (Noble & Tsien, 1968; Peper & Trautwein, 1969). Thus, the voltage ranges over which these two currents are important in determining the action potential configuration are the same: i_f is activated by hyperpolarizations to below −50 to −60 mV from holding potentials near −40 mV, as one would also observe for i_{K_2} with the same protocol, since the activation variable for i_{K_2}, s_∞, is unity at about −50 mV and zero at about −90 mV under normal conditions (Noble & Tsien, 1968).

Besides their dependence on voltage, the responses of i_f and i_{K_2} to adrenaline appear to be similar. The large and reversible inward increase of i_f observed in adrenaline during a hyperpolarizing voltage-clamp pulse from a holding potential near −40 mV (Brown *et al.* 1979; Brown & DiFrancesco, 1980) resembles the larger i_{K_2} decay that adrenaline, by shifting the s_∞ curve to more positive potentials (Hauswirth, Noble & Tsien, 1968), will produce when a similar voltage-clamp protocol is applied to Purkinje fibres. In the absence of kinetic analysis of i_f with and without adrenaline it is not yet possible to compare the effects of adrenaline on the activation curves of i_f and of i_{K_2}.

Sodium removal is known to have a depressing effect on i_{K_2}: the fully activated I–V relationship, $\bar{\imath}_{K_2}$, is reduced approximately 1·5-fold when 75% external Na is replaced by choline (DiFrancesco & McNaughton, 1979, Fig. 14) while it completely disappears in Na-free solutions (McAllister & Noble, 1966). The experimental results in Figs. 1 and 2 show that i_f is Na-dependent too: removal of 50% Na markedly

reduces the i_f time-dependent change (Fig. 1) while in 25 % Na (Fig. 2) the i_f current change disappears (or may be reversed). The similarity with i_{K_2} is therefore evident, even if in the absence of a detailed analysis of the i_f kinetics it is difficult to quantify the relation between Na concentration and corresponding amount of i_f activated by a pulse.

Furthermore, both currents are blocked by Cs. 20 mM-Cs rapidly and completely blocks i_f, as is shown in Figs. 3 and 4. Caesium is known to block the inward-rectifying K channel in skeletal muscle (Gay & Stanfield, 1977) and to reduce K permeability in the starfish egg (Hagiwara *et al.* 1976), eel electroplaque (Ruiz-Manresa *et al.* 1970) and squid axon (Adelman & French, 1978). It has also been recently shown to block the K channels i_{K_1} and i_{K_2} in sheep Purkinje fibres (Isenberg, 1977). In the experiment shown in Fig. 3, a 2 min perfusion with 20 mM-Cs was enough to cause a complete block of i_f which, as is seen in Fig. 4, extends over the whole voltage range within which i_f has been investigated.

The current i_f displays a relatively high dependence on temperature. From the data of Fig. 6, the Q_{10} values for the time constant of i_f activation are about 4·0 and 2·1 at -55 and -65 mV respectively. In another experiment (not shown) a value of 3·11 has been found at -47 mV for a similar temperature range. These values are not as great as those of 6 found by Noble & Tsien (1968) or 17 found by Cohen *et al.* (1976) for τ_s, which seems to argue against a close similarity between the two currents in this respect. However, values of Q_{10} for the time constant have to be considered a poor indication of the actual value of the activation energy for the physical process underlying the current change during a voltage clamp. This is particularly true if (as is likely to occur), the activation energy itself has a temperature dependence, even if small (S. Kroll and D. Noble, personal communication). It is worth noting that a Q_{10} of 3 is a common finding in channel-gating processes (Hodgkin & Huxley, 1952; Almers, 1972). With the relatively short pulses of Fig. 5, a temperature change causes a very large change in the amount of i_f activated. Similarly, in the beating tissue the amount of i_f activated during the repolarization process will depend sharply on the temperature and this is probably part of the mechanism whereby temperature controls the frequency of spontaneous oscillation in the S-A node.

Evidence that i_f activated during hyperpolarization cannot be a decaying outward K current

Although there are many similarities between the i_f current system in the S-A node and the i_{K_2} current system in the Purkinje fibre, the i_f current change during a hyperpolarizing clamp pulse does not seem to conform to the behaviour of a decaying K current. In normal K concentration, it is not possible to reverse i_f even at potentials more negative than the expected K equilibrium potential.

The use of very high K concentrations avoids the possible interference of K depletion in close extracellular spaces (DiFrancesco, Ohba & Ojeda, 1979) and thus the time course of i_f during hyperpolarizations in 48 mM-K, where it is still an increasing inward change (Figs. 8 and 11) provides more conclusive evidence that i_f is not carried by K.

More evidence about the nature of i_f comes from the conductance measurements made in the experiments shown in Figs. 9, 10 and 11. These demonstrate that the

onset of i_f during a clamp pulse is associated with an increase of conductance with time and therefore with a channel-opening process. The failure of Cs to decrease the initial current jump at the onset of a hyperpolarization, under conditions in which i_f is fully blocked, also favours the view that i_f is an inward current activated at potentials more negative than -40 mV. The many similarities between i_f and i_{K_2} contrast with the evidence that during a hyperpolarizing pulse i_f is a developing inward current, while i_{K_2} has always been considered to be a decaying K current (Noble & Tsien, 1968). However, very recent results which are leading to a re-consideration of the ionic nature of i_{K_2} suggest that the apparent differences between i_f and i_{K_2} can be reconciled (DiFrancesco, 1980*a*; DiFrancesco & Noble, 1980).

The data here presented do not enable us to identify the ionic mechanism underlying the current i_f. Since the possibility that i_f is a pure K current has been excluded, the Na removal experiments and the conductance measurements could suggest that i_f is carried by Na^+ ions. The effects of high K (increases in i_f) cannot, however, be directly incorporated in this simple scheme. The sensitivity to Cl of the steady-state current negative to -40 mV (Seyama, 1979) would support the view that this component could be at least partly carried by Cl-ions, but dependence of channel conductance on an external ion cannot be considered as conclusive evidence that that ion is the only one to be carried through that channel. For example, the sensitivity to Cl of the 'transient outward' current in the Purkinje fibre (Vitek & Trautwein, 1971; Fozzard & Hiraoka, 1973) does not preclude K from a role in carrying this current, given the effect of Cl on membrane permeability (Carmeliet & Verdonck, 1977). It is evident that the effect of K and Na on i_f shown in this paper require a model more complex than a simple Cl channel.

Note added in proof. Further evidence is now being obtained (DiFrancesco, 1981) that i_{K_2} in Purkinje fibres behaves not as a pure potassium current but as an inward current activated by hyperpolarizations into the pace-maker range and that both it and the current i_t in sino-atrial node are carried by sodium and potassium ions (DiFrancesco, 1980*b*; Brown, Kimura & Noble, 1980). This resolves the apparent contradiction of two cardiac currents having very similar properties but yet carried by different ions.

BROWN, H. F., KIMURA, J. & NOBLE, S. J. (1980). Evidence that the current i_t in sino-atrial node has a potassium component. *J. Physiol.* **308**, Proceedings (in the Press).

DIFRANCESCO, D. (1980*a*). The pace-maker current 'i_{K_2}' in Purkinje fibres is carried by sodium and potassium. *J. Physiol.* **308**, Proceedings (in the Press).

DIFRANCESCO, D. (1981). A new interpretation of the pacemaker current i_{K_2} in Purkinje fibres. *J. Physiol.* (in the Press).

We would like to thank H. F. Brown, A. Ferroni, D. Eisner and D. Noble for their helpful comments on the manuscript, and the C.N.R. (Italy) and the University of Milano for financial support.

REFERENCES

ADELMAN, W. J. & FRENCH, R. J. (1978). Blocking of the squid axon potassium channel by external caesium ions. *J. Physiol.* **276**, 13–25.

ALMERS, W. (1972). The decline of potassium permeability during extreme hyperpolarization in frog skeletal muscle. *J. Physiol.* **225**, 57–83.

BAUMGARTEN, C. M. & ISENBERG, G. (1977). Depletion and accumulation of potassium in the extracellular clefts of cardiac Purkinje fibres during voltage clamp hyperpolarization and depolarization. *Pflügers Arch.* **368**, 19–31.

BEAUGÉ, L. A., MEDICI, A. & SJODIN, R. A. (1973). The influence of external caesium ions on potassium efflux in frog skeletal muscle. *J. Physiol.* **228**, 1–11.

BROWN, H. F. & DIFRANCESCO, D. (1980). Voltage-clamp investigations of membrane currents underlying pace-maker activity in rabbit sino-atrial node. *J. Physiol.* **308**, 331–351.

BROWN, H. F., DIFRANCESCO, D. & NOBLE, S. J. (1979). How does adrenaline accelerate the heart? *Nature, Lond.* **280**, 235–236.

CARMELIET, E. & VERDONCK, F. (1977). Reduction of potassium permeability by chloride substitution in cardiac cells. *J. Physiol.* **265**, 193–206.

COHEN, I., DAUT, J. & NOBLE, D. (1976). The effects of potassium and temperature on the pace-maker current, i_{K_2}, in Purkinje fibres. *J. Physiol.* **260**, 55–74.

DIFRANCESCO, D. (1980). Could i_{K_2} in Purkinje fibres be an inward current activated on hyperpolarization? *J. Physiol.* **305**, 64*P*.

DIFRANCESCO, D. & MCNAUGHTON, P. A. (1979). The effects of calcium on outward membrane currents in the cardiac Purkinje fibre. *J. Physiol.* **289**, 347–373.

DIFRANCESCO, D. & NOBLE, D. (1980). If i_{K_2} is an inward current, why does it display potassium specificity? *J. Physiol.* **305**, 14*P*.

DIFRANCESCO, D., NOMA, A. & TRAUTWEIN, W. (1979). Kinetics and magnitude of the time-dependent potassium current in the rabbit sino-atrial node. *Pflügers Arch.* **381**, 271–279.

DIFRANCESCO, D., OHBA, M. & OJEDA, C. (1979). Measurement and significance of the reversal potential for the pace-maker current (i_{K_2}) in sheep Purkinje fibres. *J. Physiol.* **297**, 135–162.

FOZZARD, H. A. & HIRAOKA, M. (1973). The positive dynamic current and its inactivation properties in cardiac Purkinje fibres. *J. Physiol.* **234**, 569–586.

GAY, L. A. & STANFIELD, P. R. (1977). Cs^+ causes a voltage-dependent block of inward K-currents in resting skeletal muscle fibres. *Nature, Lond.* **267**, 169–170.

HAGIWARA, S., MIYAZAKI, S. & ROSENTHAL, N. P. (1976). Potassium current and the effect of cesium on this current during anomalous rectification of the egg cell membrane of a starfish. *J. gen. Physiol.* **65**, 621–638.

HAGIWARA, S. & TAKAHASHI, K. (1974). The anomalous rectification and cation selectivity of the membrane of a starfish egg cell. *J. Membrane Biol.* **18**, 61–80.

HAUSWIRTH, O., NOBLE, D. & TSIEN, R. W. (1968). Adrenaline: mechanism of action on the pace-maker potential in cardiac Purkinje fibres. *Science, N.Y.* **162**, 916–917.

HODGKIN, A. L. & HUXLEY, A. F. (1952). A quantitative description of membrane current and its application to conduction and excitation in nerve. *J. Physiol.* **116**, 497–506.

ISENBERG, G. (1977). Cardiac Purkinje fibres: cesium as a tool to block inward rectifying potassium currents. *Pflügers Arch.* **365**, 99–106.

MCALLISTER, R. E. & NOBLE, D. (1966). The time and voltage dependence of the slow outward current in cardiac Purkinje fibres. *J. Physiol.* **186**, 632–662.

NOBLE, D. & TSIEN, R. W. (1968). The kinetics and rectifier properties of the slow potassium current in cardiac Purkinje fibres. *J. Physiol.* **195**, 185–214.

PEPER, K. & TRAUTWEIN, W. (1969). A note on the pace-maker current in Purkinje fibres. *Pflügers Arch.* **309**, 356–361.

RUIZ-MANRESA, F., RUARTE, A. C., SCHWARTZ, T. L. & GRUNDFEST, H. (1970). Potassium inactivation and impedance changes during spike electrogenesis in eel electroplaques. *J. gen. Physiol.* **55**, 33–47.

SEYAMA, I. (1979). Characteristics of the anion channel in the sino-atrial node cell of the rabbit. *J. Physiol.* **294**, 447–460.

VITEK, M. & TRAUTWEIN, W. (1971). Slow inward current and action potentials in cardiac Purkinje fibres. *Pflügers Arch.* **323**, 204–218.